

IN VITRO ANTIFUNGAL ACTIVITY OF *Chromolaena odorata* (L.) R.M. King & H. Rob (Siam Weed) AND *Senna alata* L. (Candle bush) BIHERBAL METHANOLIC EXTRACT AGAINST *Tinea capitis*

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Abstract

Tinea capitis (Scalp ringworm) is a prevalent dermatophytic infection that poses treatment challenges due to emerging antifungal resistance and the cost of orthodox medications. This study evaluated the *in vitro* antifungal efficacy of a bi-herbal methanolic extract from *Chromolaena odorata* and *Senna alata* against *Tinea capitis*. The antifungal activity was evaluated using the agar well diffusion and broth microdilution methods to determine the zones of inhibition (ZOI) and minimum inhibitory concentration (MIC). The results demonstrated a dose-dependent antifungal activity, with the highest ZOI of 18.25 ± 0.22 mm at a 0.30% w/v concentration, compared to 22.10 ± 0.18 mm for the control (ketoconazole) (0.05% w/v). The MIC was determined to be 50% w/v. The phytochemical screening showed the presence of some bioactive compounds, including high concentrations of alkaloids, phenols, and tannins, moderate levels of flavonoids, saponins, and terpenoids, as well as low level of cardiac glycosides. The findings show that the bi-herbal extract of *C. odorata* and *S. alata* has significant antidermatophytic activity and thus supports its traditional use and potential as a complementary or alternative natural therapeutic agent for managing *Tinea capitis* infections. This study recommends further research to isolate and identify the specific active compounds responsible for this activity.

Keywords: *Tinea capitis*, dermatophyte, bi-herbal, antifungal

Introduction

Dermatophytes are a common label for a group of fungus of Arthrodermataceae that commonly cause skin disease in animals and humans (Martinez-Rossi *et al.*, 2021).

Traditionally, these anamorphic (asexual or imperfect fungi) mold genera are: Microsporum, Epidermophyton and Trichophyton (Al-Khikani, 2020). There are about 40 species in these three genera. Species capable of reproducing sexually

belong in the teleomorphiogenic Arthroderma, of the Ascomycota. As of 2019 a total of nine genera is identified and new phylogenetic taxonomy has been proposed (Al-Khikani, 2020).

Dermatophytes are the causative agents of Dermatophytosis (also known as tinea or ringworm infection) (Ameen, 2010). These fungal species are associated with the stratum corneum of the epidermis and keratinized tissues such as skin, hair and nails of humans and animals causing skin infection which ranked as one of the most common cutaneous conditions all over the world (Ameen, 2010; Ndako *et al.*, 2012). A group of closely related fungi comprising of 40 identified species in the dermatophytic genera that include Trichophyton, Microsporum and Epidermophyton are documented in literature as potential etiological agents of skin infection including dermatophytosis (Adefemi *et al.*, 2011).

It is a contagious fungus which primarily causes dermatophytosis such as *Tinea pedis*, *Tinea unguium*, *Tinea corporis*, and *Tinea capitis* (Petrucelli *et al.*, 2020). It distributes all over the world, especially in moist and carbon- rich environments. While *T. rubrum* is the most prevalent dermatophyte isolated from humans accounting for about 80% of dermatomycoses that affect keratinized

tissues such as skin, hair, and nails (Gupta and Versteeg, 2020). It is a worldwide agent responsible for chronic cases of dermatophytosis which have high rates of resistance to anti- fungal drugs. Moreover, infections due to *T. rubrum* are often associated with relapses following cessation of anti- fungal therapy (de Oliveira-Pereira *et al.*, 2013).

Among the following pathogens of the skin, the most conspicuous groups are species of candida and dermatophytes. Dermatophytosis is the most ubiquitous and contagious fungi disease in humans, and it is engendered with dermatophytes invade keratinized tissues of humans (Ghannoum *et al.*, 2010). The prevalence of dermatophytosis has witnessed a geometric progression in the past decade (Kruithoff *et al.*, 2023). Besides high humidity, poor hygiene and overcrowding are conditions suited for the growth of dermatophytes. And the penultimate and latter conditions are endemic in Nigeria (Zanna *et al.*, 2021). Common therapeutic strategies for dermatophytosis are based on the use of tropical and systemic anti- fungal agents (Ahmed *et al.*, 2025). The present line of treatment includes tolnaftate, terbinafine hydrochloride and imidazoles such as

ketoconazole, miconazole nitrate and clotrimazole (Rotta *et al.*, 2016).

Despite an increasing number of safe and highly effective agents available for the treatment of dermatophytosis, there are reports suggesting possible resistance of dermatophytes to anti-fungal agents (Martinez-Rossi *et al.*, 2021). Furthermore, fungal infections need long-term therapy involving several weeks. However, the patients discontinue the application, due to cost factor, resulting in the recurrence of the disease. Therefore, there exists a clear demand for additional anti-fungal with therapeutic potentials. Hence, herbal plants products that inhibit their growth without harming the host represent the potential therapeutic agents. This study introduced the use of methanolic extract from two plants in an attempt to demonstrate their anti-dermatophytic activity human fungal disorder caused by dermatophytes. This study aims to evaluate the efficacy of a Bi-herbal made from (*Chromolaena odorata* and *Senna alata*) in the controls of a human Dermatophytic fungi (*Tinea corporis*).

Materials and Methods

Materials and Reagents

Materials for the analysis include fresh leaves of *Senna alata* and *Chromolaena odorata*, incubator, autoclave, microscope, refrigerator and weighing balance. Reagents required for the experiment includes disposable petri dish, Nutrient Agar, Potato Dextrose Agar, Crystal violet stain, Acetone, Safranin and Hydrogen peroxide.

Sterilization of Glassware and Laboratory Benches

All glasses including petri-dishes, beaker, test tube were thoroughly washed with detergents and rinsed severally with distilled water, allowed to dry, wrapped up in an aluminium foil paper and sterilized in an autoclave at pressure of 15psi (Singleton and Sainsbury, 1995). Forceps, cork borer and blade were sterilized with 70% ethanol and then heated to red hot and allowed to cool (Collins *et al.*, 2004). The various media used were also sterilized in the autoclave at 121⁰C for 15minutes (Atlas *et al.*, 2010). The laboratory bench was cleaned with ethanol, Izal, Dettol and jik.

Source and Identification of Plant Material

Fresh leaves of *Chromolaena odorata* and *Senna alata* were collected from Benin City Edo, State. The plants were authenticated at

the Herbarium Unit, Department of Botany, Delta State University, Abraka with Voucher Numbers DELSUH-270 (*Chromolaena odorata*) and DELSUH-217 (*Senna alata*)

Fungi Culture

The fungus that was used for the study was *Tinea capitis* and obtained from stock cultures at the Agbonlahor Research Center, Ikpoba Hills, Benin City. The fungus was grown aerobically on Mueller- Hinton agar (BBL, USA) at 37⁰C using the methods of Abdul *et al.* (2010). The fungi were preserved in tryptic soy broth (BBL, USA) + 10% (V/V) glycerol at -80⁰C prior to revival. In each case they were revived shortly before antifungal assay.

Preparation of the Bi-herbal extract

The extraction and preparation of the plant materials and Bi-herbal formulation were carried out following the procedure described by Ononamadu *et al.* (2020) with modifications. The fresh leaves of the plants were collected and thoroughly rinsed under running tap water to remove soil and dust particles. The clean leaves were then air-dried at room temperature in the laboratory for 15 days. After drying, the leaves were grounded into fine powder using a mechanical grinder. Five (5) grams of each

powdered sample were soaked in 50 mL of methanol for 24 hours using an orbital shaker at ambient temperature. The resulting mixture was filtered through Whatman No. 1 filter paper, and the filtrate was concentrated using a rotary evaporator. The concentrated extract was then stored in an airtight container at 4°C for subsequent analysis.

Preparation for Treatment Solution

The treatment solutions were prepared following the procedure described by Adeyemo *et al.* (2018), using the bi-herbal extract at varying concentrations based on percentage weight. To obtain the stock solution, 100 mL of distilled water was added to 100 g of the concentrated biherbal extract and allowed to stand overnight, yielding a 100% biherbal extract (1.1 w/v). Subsequently, aqueous solutions of different concentrations (25%, 50%, and 75%) were prepared by serially diluting the stock extract with distilled water.

Phytochemical Screening

The Qualitative phytochemical analysis of the ethanolic extract was conducted using standard procedures by Sharififar *et al.* (2013) to identify the presence of major bioactive compounds, as outlined below:

Determination of Alkaloids

0.5 g of the extract was stirred with 5 ml of 1% aqueous HCl on a steam bath and filtered. The filtrate was treated with a few drops of Dragendorff's reagent (Sharififar *et al.*, 2013). Turbidity or precipitation indicated a positive result.

Determination of Saponin content

0.5 g of the extract was dissolved in 5 ml of distilled water and shaken vigorously. The formation of a persistent froth upon warming, which formed an emulsion with olive oil, was taken as a positive test (Sharififar *et al.*, 2013).

Determination of Tannin content

0.5 g of the extract was boiled in 10 ml of water and filtered. A few drops of 0.1% ferric chloride were added to the filtrate. A brownish-green or blue-black coloration confirmed the presence of tannins (Sharififar *et al.*, 2013).

Determination of Anthraquinones

0.5 g of the extract was boiled with 10 ml of sulphuric acid and filtered. The filtrate was shaken with 5 ml of chloroform, and the chloroform layer was separated. The addition of 1 ml of 10% ammonia solution resulting in a color change indicated the presence of anthraquinones (Sharififar *et al.*, 2013).

Determination of Flavonoid content

Five (5) ml of a dilute aqueous filtrate of the extract was treated with 1 ml of concentrated sulphuric acid. The appearance of a yellow coloration that disappeared on standing was

considered a positive test (Sharififar *et al.*, 2013).

Determination of Cardiac Glycosides (Keller-Killiani Test)

0.5 g of the extract was diluted with 5 ml of water. Then, 2 ml of glacial acetic acid containing one drop of ferric chloride solution was added. This was underlaid with 1 ml of concentrated sulphuric acid. A brown ring at the interface indicated the presence of deoxysugar characteristic of cardenolides (Sharififar *et al.*, 2013).

Determination of Phenolic contents

Total phenolic content was determined using the Tannic Acid Equivalent (TAE) assay, which measures the relative astringency of the extract (Sharififar *et al.*, 2013).

Antifungal Activity by Agar Well Diffusion Method

The antidermatophytic activity of the bi-herbal extract was evaluated using the agar well diffusion technique (Jorgensen and Ferraro, 2009). Potato Dextrose Agar (PDA) plates were uniformly inoculated with 1 mL of a dermatophyte suspension standardized to 1×10^7 SFU/mL. Wells of 8 mm diameter were aseptically punched into the agar using a sterile cork borer. Different concentrations (0.05%, 0.10%, 0.20%, and 0.30% w/v) of the bi-herbal extract were prepared and carefully dispensed into the wells.

Ketoconazole (0.05% w/v) was used as the standard positive control. The inoculated plates were then incubated at 25°C for 5 to 7 days. After incubation, the diameters of the zones of inhibition (ZOI) surrounding each well were measured in millimeters (mm) (Jorgensen and Ferraro, 2009). All experiments were performed in triplicate, and the results were expressed as the mean ZOI \pm standard deviation.

Determination of Minimum Inhibitory Concentration (MIC)

The minimum inhibitory concentration (MIC) was determined using the broth microdilution method according to the Clinical and Laboratory Standards Institute (CLSI) M38 reference method (CLSI, 2017) with modifications. A stock solution of the bi-herbal extract was prepared at a concentration of 100 mg/mL in dimethyl sulfoxide (DMSO). This stock was subsequently diluted in sterile broth medium to a starting concentration of 1000 μ g/mL and subjected to a two-fold serial dilution in a 96-well microplate to achieve a final concentration range of 1000 μ g/mL to 7.8

μ g/mL. Each well was inoculated with a standardized dermatophyte suspension to a final concentration of 5×10^5 SFU/mL. The microplate included the following controls: sterile medium (sterility control), medium with inoculum (growth control), and Ketoconazole (0.1% w/v) as a positive antifungal control. The plates were sealed and incubated at 28°C for 5-7 days. The MIC was defined as the lowest concentration of the extract that resulted in at least 80% inhibition of fungal growth, as visually compared to the growth control well (CLSI, 2017).

Statistical Analysis

The data obtained from the study were analyzed using SPSS version 31 and Graph Pad Prism 9. The results were presented as Mean \pm Standard deviation and the means were separated using Turkey's Post Hoc Test.

Results

Minimum inhibitory concentration of the Bi-Herbal extract

The data indicate that the extract exhibited inhibitory activity at concentrations of 100%, 75%, and 50% and that of the control, while no inhibitory effect was observed at 20%.

Table 1: Minimum inhibitory concentration extract of bi-herbal extract against *Tinea capitis*

Test organisms	Control (Ket.)	100	75	50	20	MIC (w/v%)
<i>Tinea capitis</i>	-	-	-	-	+	50

Interpretation

+ = Growth

- = No growth

Antifungal activity of the Bi-herbal extract against *Tinea capitis*

The results presented in Table 2 showed that the bi-herbal extract of *Chromolaena odorata* and *Senna alata* exhibited antifungal activity against *Tinea capitis*, with inhibition zones increasing proportionally with extract concentration. The highest activity was

observed at 0.30% w/v, producing a mean inhibition zone of 18.25 ± 0.22 mm, while the lowest inhibition (8.70 ± 0.15 mm) occurred at 0.05% w/v. Although the standard antifungal agent, ketoconazole (0.05% w/v), produced the highest inhibition zone (22.10 ± 0.18 mm), the performance of the bi-herbal extract, especially at 0.30% was comparably high.

Table 2: Antifungal Activity of the Crude Biherbal Extract of *Chromolaena odorata* and *Senna alata*

Isolate	0.30% w/v	0.20% w/v	0.10% w/v	0.05% w/v	Ket. 0.05% w/v
<i>Tinea capitis</i>	18.25 ± 0.22	15.60 ± 0.25	12.45 ± 0.20	8.70 ± 0.15	22.10 ± 0.18

*Values are presented as Mean \pm Standard deviation (n=5)

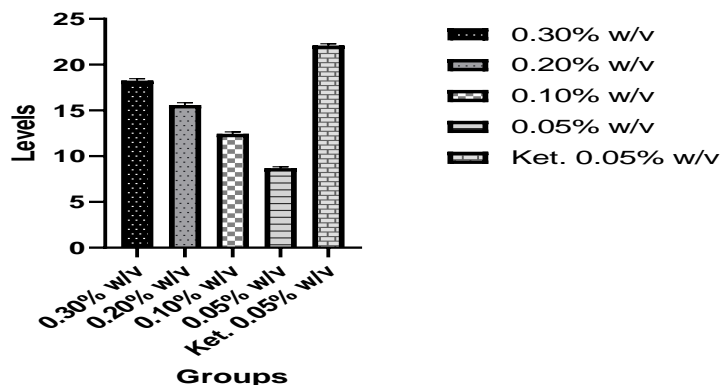


Fig 1: Antifungal Activity of the Bi-herbal extract of *Chromolaena odorata* and *Senna alata*. Bars represent Mean \pm Standard deviation (n=5), and the means were separated using Turkey's post Hoc test.

Phytochemical composition of the Bi-herbal extract

The phytochemical screening of the bi-herbal extract of *Chromolaena odorata* and *Senna alata* (Table 3) revealed the presence of

several bioactive secondary metabolites, such as alkaloids, phenols, and tannins in high concentrations, with moderate levels of flavonoids, saponins, and terpenoids, and a low presence of glycosides.

Table 3: Phytochemical screening of Biherbal extract

Phytochemicals	Inference
Alkaloids	+++
Glycosides	+
Flavonoids	++
Phenols	+++
Saponins	++
Tannins	+++
Terpenoids	++

Keys: + = low, ++ moderate, +++ = High

Discussion

The bi-herbal extract inhibited fungal growth at concentrations of 100%, 75%, and 50%, while no inhibition was observed at 20% (Table 1). This shows that higher concentrations of the bi-herbal formulation are more effective. The standard antifungal drug, ketoconazole (Positive control), also showed strong inhibitory activity. The inhibitory effect of the bi-herbal extract that was observed aligns with reports that plant-based formulations can exhibit comparable efficacy to synthetic antifungal drugs (Sule *et al.*, 2011).

These findings are consistent with earlier studies on the antifungal properties of *Senna alata* and *Chromolaena odorata*, which are the constituent plants of the bi-herbal mixture. Ngane *et al.* (2006) reported that *C. odorata* extracts exhibited MIC values ranging between 62.5 and 500 µg/mL against

dermatophytes, depending on solvent polarity, while Sule *et al.* (2011) found that *S. alata* leaf extracts showed strong inhibitory activity against *Candida albicans* and *Trichophyton* species. Similarly, Kaur *et al.* (2021) made emphasis that crude plant extracts typically require higher concentrations to achieve inhibition compared to synthetic drugs, a finding that corresponds with the relatively high MIC observed in this study. Savarirajan *et al.* (2021) observed that antifungal potency in plant extracts is strongly influenced by extraction methods and phytochemical composition, suggesting that the activity of this bi-herbal extract may be attributed to synergistic effects between secondary metabolites such as flavonoids, saponins, and phenols. Overall, the results confirm that the bi-herbal extract possesses notable antifungal potential and could serve as a natural alternative for managing dermatophytic infections such as *Tinea capitis*.

Table 2 showed that the extract demonstrated strong antifungal activity against *Tinea capitis*, with the inhibition zone decreasing from 18.25 ± 0.22 mm at 0.30% w/v to 8.70 ± 0.15 mm at 0.05% w/v, while ketoconazole (0.05% w/v) recorded 22.10 ± 0.18 mm. This dose-dependent inhibition agrees with the findings of Agré *et al.*, (2024), who observed that extracts of *Ocimum gratissimum* and *Azadirachta indica* inhibited *Microsporum canis* and *Epidermophyton floccosum* in a similar concentration-dependent pattern. Although the standard antifungal drug (ketoconazole) exhibited slightly higher activity, the inhibitory potential of the bi-herbal extract remains noteworthy, particularly considering its natural origin and potential affordability.

The phytochemical profile presented in Table 3 shows high levels of alkaloids, phenols, and tannins, with moderate quantities of flavonoids, saponins, and terpenoids, and a low presence of glycosides. This composition supports the antifungal efficacy observed, since these bioactive constituents are known to contribute significantly to antifungal mechanisms. For instance, phenolic compounds and tannins interfere with fungal cell wall synthesis and protein precipitation, leading to cell death (Curatolo *et al.*, 2021). Flavonoids and alkaloids have been reported to disrupt fungal membranes and inhibit spore germination (Sharma *et al.*, 2025). These findings suggest that the antifungal potential of the extract is likely due to the synergistic action of these phytochemicals.

While the bi-herbal extract did not surpass ketoconazole in potency, its near-comparable inhibition zone (18.25 mm versus 22.10 mm)

demonstrates significant potential for alternative or complementary use in managing dermatophytic infections. The observed synergy between *Chromolaena odorata* and *Senna alata* may have enhanced the extract's efficacy, as previous studies reported improved antifungal potency in combined herbal formulations (Agre *et al.*, 2024; Omer *et al.*, 2023).

Conclusion

This study shows that bi-herbal extract of *Chromolaena odorata* and *Senna alata* exhibit antifungal effects against dermatophytic fungi *Tinea capitis*. However, at low concentrations, these species showed no antifungal activity.

Recommendations

Further studies are needed to determine the chemical identity of the bioactive compounds responsible for the observed antifungal activity. Natural plant-derived fungicides may be a source of new alternative active compounds, in particular with antifungal activity. The high proportion of active extracts in the assayed species, selected according to available ethnobotanical data, corroborates the validity of this approach for the selection of plants species in the search for a specific activity.

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